sensitivity for very low quantities of analyte. All three independent claims 1, 23, and 26 require: a microscopic size of the sorbent zones, a substantial depletion of analyte from the sample, and a concentration of the depleted analyte on the microscopic sorbent zones.

The Examiner appears to believe that it would have been obvious to use the technique to allow for analyte depletion in a sample as taught by Figure 4 of the Immunoassay reference in the binding assay of the '202 patent that teaches using small amounts of the binding agents. The Examiner further argues that because the '202 patent recognizes the use of small amounts of the binding agents, it would be feasible to place the binding agent on a very small area of a solid support because "[a] high coating density is generally desirable to maximize signal/noise ratios" and to arrive at the present invention. Applicants respectfully disagree. As explained in detail in the Declaration by Dr. Silzel (filed concurrently herewith), a co-inventor, and contrary to the Examiner's view, the concept of substantial depletion of an analyte from the solution and its concentration onto the microscopic sorbent zones is not obvious in view of the cited references.

First, Figure 4 does not teach or suggest a substantial depletion of an analyte under non-equilibrium conditions and represents a purely theoretical and generally known equilibrium model for a first-order binding process. Figure 4 does not convey any new information, but simply shows that theoretically, given enough binding partner and enough time, one can bind any amount of analyte. However, as explained in Section 6 of the Declaration, in the present invention, analyte binding is not a first-order process and it occurs under non-equilibrium conditions. Accordingly, Figure 4 does not accurately reflect non-equilibrium and non-first order analyte binding by microscopic sorbent zones occurring in the instant invention. Therefore, Figure 4 does not teach substantial depletion of an analyte by microscopic sorbent zones.

Second, contrary to the Examiner's belief, prior to the present invention, a high coating density was not desirable in either analyte-depleting microplate assays

or Ekins' ambient analyte assay. As explained in Section 7 of the Declaration, when the coating density of the binding partner is increased, multiple layers of the binding partner may form. Prior to the present invention, it was generally understood in the art that when such multiple layers are formed, the overall binding capacity of the binding partner substantially decreases due to "blocking" of the lower layers by the top layers.

Moreover, the '202 patent teaches away from an increase in coating density. The '202 patent explicitly states that the invention "involves the realization that the use of high qualities of binding agent is neither necessary for good sensitivity in immunoassays nor is it generally desirable" (page 3, lines 28-29). Thus, the '202 patent teaches against an increase of coating density. Therefore, based on this teaching of the '202 patent and in view of the general state in the art prior to the present invention, those skilled in the art would have been at least discouraged from increasing the coating density of the binding agent on the microscopic binding zones of the '202 patent as suggested by the Examiner to arrive at the present invention.

Third, the concept of a substantial analyte depletion from the solution and concentration of the analyte onto the microscopic sorbent zones is not obvious in view of the cited references because it is based on an unexpected discovery. The concept is based on a discovery that the binding capacity of a conventional microplate well may be concentrated onto an area of a binding zone that is 100 times smaller than that of the microplate well without a significant loss of analyte-depletion capability.

In the present invention, about $10^{10}$ molecules of binding partner are immobilized per each sorbent zone (*see* Example 1 of the present invention) resulting in a three-dimensional matrix about 3 monolayers deep (*see*, for example, page 19, lines 16-31, of the instant specification discussing irregular topology of the immobilized binding-partner molecules extending up to 200 nm vertically from the

surface of the film). As discussed in Section 7 of the Declaration, those skilled in the art would not have expected that such a three-dimensional matrix is possible without "blocking" analyte access to the binding-partner molecules on the lower layers.

However, the present invention demonstrates the functionality and accessibility of the binding partner throughout the three-dimensional matrix. As explained in the specification, this result is achieved by using a photo-linking technique and printing antibody at concentrations 1000 times greater than the 1 μM solutions typically used to coat microplates (page 17, lines 18-32). Therefore, it was unexpected by those skilled in the art that a three-dimensional cross-linked matrix of binding-partner molecules of the present invention allows the same level of analyte binding as a two-dimensional surface of a microplate well.

Additionally, prior to the instant invention, it was not expected in the art that microscopic sorbent zones in contact with conventional sample volumes are capable of harvesting substantially the same amount of an analyte from the sample within the same incubation time as the equivalent amount of the antibody spread across a microplate well or distributed in the sample solution. As explained in Section 7 of the Declaration, such a result was unexpected given the complexities of the non-equilibrium conditions, cooperativity in binding, diffusion, local concentration effects, and other nonlinear effects occurring when an analyte is concentrated onto the microscopic sorbent zones.

Fourth, the concept of substantial analyte depletion from the solution and its concentration onto the microscopic sorbent zones is not obvious in view of the cited references because it produces unexpected results. As explained in Section 9 of the Declaration, in the present invention, substantially the same signal as in the conventional microplate assay is generated in a zone only a few hundred microns in diameter, and, thus, having a surface area about 100 times smaller than that of a microwell. Since background signals are roughly proportional to the viewed surface

area, the microscale assay of the present invention advantageously and unexpectedly produces a signal-to-background ratio that are orders of magnitude greater than in conventional microplate assays.

The results of the present invention are also unexpected in view of the '202 patent as explained in the previous response to the Final Office Action dated August 14, 2001. The unexpected benefit of a high signal-to-background ratio of the present invention is derived from the confinement of the binding partner to a small area of support to maximize analyte-binding capacity per unit area. For example, as explained on p. 8, lines 2-23, and as demonstrated in Figure 1 of the present invention, the mass assay of the present invention has about a 10-100 times higher analyte-binding capacity than the ambient assay of the Immunoassay reference. In Figure 1, the horizontal axis shows the molar concentration of the analyte sample applied to a microscopic zone and the vertical axis shows the number of molecules bound by a single microscopic zone per 100 µl of the sample. For each analyte concentration, an amount of the analyte bound from a 100 µl sample is calculated based on the Ambient Analyte model of Ekins and the Mass Assay model of the present invention. The resulting graphs show a 10-100 times higher binding capacity of the arrays of the present invention as compared to those of Ekins, and, thus, a 10-100 times higher sensitivity of the assay of the present invention as compared to the assay of Ekins.

Fifth, applicants also argued in the previous response that the instant invention is not obvious in view of the cited art because the assay of the present invention senses mass, whereas the Ekins assay measures analyte concentration. Applicants explained that, in the present invention, the analyte depletion is so substantial that it maximally perturbs the bulk concentration of analyte in the sample. As a result, the developed signal reflects the total analyte mass contained in the defined sample volume and harvested onto the microscopic measurement spot (page 7, lines 21-31). The Immunoassay reference, on the contrary, teaches only an insignificant and localized analyte depletion in the medium immediately surrounding the analyte-binding sites. Such minuscule analyte depletion does not

appreciably affect the overall bulk concentration of the analyte in the sample solution (page 173, left column). Accordingly, the developed signal is indicative of an ambient analyte concentration, but not the total analyte mass in the sample.

In response to this argument, the Examiner stated that the instant invention uses "the same method steps, the same laser microscopy techniques to assay the analyte, and provides results in terms of molecules bound." Applicants disagree.

As explained on pages 20, lines 11-21, line 6, the arrays of the present invention respond to analyte mass, not to concentration, because they have a sufficient affinity and binding capacity to deplete substantially all analyte contained in a sample. Figure 6 demonstrates such a mass-sensing nature of the present invention. Figure 6 shows experimental results from three-hour incubations in which 100 microliter aliquots of $5x10^{10}$M DBCY5-biotin solution were in contact with arrays having 1, 9, 25, 49, or 70 spots. When the number of avidin spots per array is varied, it is expected in a mass-sensing array that the density of analyte bound per spot would vary inversely with the number of spots present. This is exactly the result shown in Figure 6: when fewer spots are printed per array, the total analyte mass present during incubation is collected onto fewer spots, leading to an increased signal from any one spot.

Also, as discussed above, unlike the Ekins references, the binding partner of the present invention is highly concentrated in each binding zone and forms a three-dimensional matrix capable of depleting substantially all analyte from a sample. Therefore, contrary to the Examiner's belief, the present invention does provide a mass-sensing assay unlike the cited references.

In summary, Figure 4 of the Immunoassay reference does not teach or suggest a substantial depletion of the analyte from the sample and its concentration on the microscopic sorbent zones. Also, the '202 patent explicitly teaches against increasing the coating density of the binding partner and, thus, teaches away from the combination of the references suggested by the Examiner. Finally, the present invention is based on unexpected discoveries and produces unexpected results.

In light of the foregoing, applicants respectfully submit that the '202 patent and the Immunoassay reference, either alone or in combination with other known techniques of the art, cannot make claims 1, 23, and 26 obvious. None of the cited references, either alone or in combination, would have motivated one skilled in the art to arrive at the present invention, which requires a substantial depletion of the analyte from the bulk solution and concentration of the analyte on the microscopic sorbent zones. Claims 2-4, 13-19, 24, and 25-28 depend, directly or indirectly, on claims 1, 23, and 26, and are not obvious for at least the same reasons. Accordingly, withdrawal of the rejection to claims 1-4, 13-19, and 23-28 is respectfully requested.

Claims 1-4, 13-19, 21, and 23-28 are rejected under 35 U.S.C. § 103(a) as being unpatentable over the '202 patent in view of Ekins *et al.*, *Analytica Chimica Acta* (Analytica reference). This rejection is respectfully traversed.

The '202 patent and the state of the art prior to the present invention are discussed above. The Analytica reference cannot remedy the defect of the '202 patent. As discussed in our previous response to the first Office Action, similarly to the Immunoassay reference, the Analytica reference discloses an ambient analyte immunoassay. Figure 4 of the Analytica reference that allegedly teaches the depletion of analyte is identical to Figure 4 of the Immunoassay reference discussed in detail above. In light of the foregoing, applicants respectfully submit that the '202 patent and the Analytica reference, either alone or in combination, cannot make claims 1, 23, and 26 obvious. Claims 2-4, 13-19, 24, and 25 depend, directly or indirectly, on claims 1 and 23 and are not obvious for at least the same reasons. Accordingly, withdrawal of the rejection to claims 1-4, 13-19, 21, and 23-28 is respectfully requested.

Claims 5-10 are rejected under 35 U.S.C. § 103(a) as being unpatentable over the '202 patent, and either the Immunoassay reference or the Analytica reference, in further view of Ullman *et al.* (U.S. Patent 5,512,659). Claim 11 is rejected under 35 U.S.C. § 103(a) as being unpatentable over the '202 patent, and either the Immunoassay reference or the Analytica reference in further view of Waggoner *et al.* (U.S. Patent 5,368,486). Claim 12 is rejected under 35 U.S.C. § 103(a) as

being unpatentable over the '202 patent, and either the Immunoassay reference or the Analytica reference in view of Waggoner *et al.*, in further view of Lee *et al.* (U.S. Patent 5,453,505). Claim 20 is rejected under 35 U.S.C. § 103(a) as being unpatentable over the '202 patent, and either the Immunoassay reference or the Analytica reference in view of Northrup *et al.* (U.S. Patent 5,639,423). Applicants respectfully traverse these rejections.

As discussed above, the '202 patent, the Immunoassay reference, and the Analytica reference, either alone or in combination, cannot make claim 1 obvious, because they do not teach or suggest the binding assay of the present application, which requires the analyte to be substantially depleted from the sample and concentrated on microscopic sorbent zones. Claims 5-10, 11, 12, and 20 depend, directly or indirectly, from claim 1 and cannot be made obvious by the '202 patent, the Immunoassay reference, and the Analytica reference for at least the same reasons.

Ullman *et al.*, Waggoner *et al.*, Lee *et al.*, and Northrup *et al.* cannot remedy the defect of the '202 patent, the Immunoassay reference, and the Analytica reference, and are not relied upon by the Examiner for such. Ullman *et al.*, Waggoner *et al.*, Lee *et al.*, and Northrup *et al.* have no teaching whatsoever of a binding assay utilizing a plurality of sorbent zones containing an analyte-binding partner, let alone a binding assay, which requires an excess of the analyte-binding partner relative to the analyte, so that any analyte present is substantially depleted from the sample and concentrated in the sorbent zones. Therefore, none of the cited references, either alone or in combination, can motivate one skilled in the art to arrive at claims 5-10, 11, 12, and 20. Withdrawal of the rejection is, therefore, respectfully requested.

In view of the foregoing, it is respectfully submitted that the application is in condition for allowance. Reexamination and reconsideration of the application are requested.

If for any reason the Examiner finds the application other than in condition for allowance, the Examiner is requested to call the undersigned attorney at the Los Angeles, California, telephone number 213-337-6700 to discuss the steps necessary for placing the application in condition for allowance.

Respectfully submitted,

HOGAN & HARTSON L.L.P.

Date: 5/13, 2002

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